Abstract

MDS and AML are malignancies characterized by malignant cell expansion and suppression of normal hematopoietic activity due to an inflammatory cytokine milieu in the marrow. Since cytokines are a major source of morbidity, there is a need for therapeutics that can target the malignant clones while also relaxing the repression of healthy hematopoietic stem cells. We determined that expression of Angiopoietin-1 (Ang-1) was significantly elevated in a large cohort of MDS/SAH stem cells (n=1381) and that higher expression was associated with higher transfusion requirements in patients. Higher Angiopoietin-1 expression was also found to be associated with reduced overall survival in MDS as well as in AML cohorts. The receptor for Angiopoietin-1, Tie-2, was also significantly elevated in MDS stem cells and its knockdown led to inhibition of leukemic cell proliferation, demonstrating its utility as a therapeutic target. Next, we tested the efficacy of ARRY-614, a novel specific inhibitor of Tie-2 as well as p38 MAPK, a kinase that has been shown to be over activated in MDS. ARRY-614 inhibited p38 MAPK and abrogated the activation of downstream effectors kinases Mapkak2 and Elf4e in leukemic cells and led to decreased leukemic cell growth. ARRY-614 treatment was able to inhibit TNF-a mediated inhibitory effects on normal hematopoietic stem cells. Importantly, treatment of primary MDS samples with the dual inhibitor led to stimulation of erythroid and myeloid colonies. These data demonstrate that dual inhibition of Tie-2 and p38 MAPK in MDS/AML can inhibit malignant cell growth and reverse inhibitory effects of cytokines on healthy hematopoietic.

Introduction

- AML and MDS are clonal hematopoietic disorders manifesting as cytopenias or hyperleukocytosis at the extremes of clinical spectrum. These disorders are associated with significant morbidity and mortality.
- Cytopenias are a major source of morbidity with bleeding, infections and need for repeat transfusion that could lead to unbearable long term sequel.
- Various inflammatory cytokines like Tumor necrosis factors (TNF-a), Transforming growth factor (TGF-b) and interleukins are implicated in pathogenesis of cytokines associated with MDS and AML.
- Angiopoietins-1 is a cytokine involved in angiogenesis and other processes, effects mediated by binding to receptor tyrosine kinase Tie-2.
- Tie-2 in murine models promotes stem cell quiescence and self-renewal.
- Tie-2 is also enriched in leukemic cells that are resistant to chemotherapy and participates in the cross-talk between the microenvironment and malignant clones.
- Since AML/MDS initiating stem cells are associated with high rates of self renewal and differentiation block, we investigated the role of Ang-1/Tie-2 in these malignancies.
- p38 mitogen activated protein kinase (MAPK) is an evolutionary conserved serine-threonine kinase involved in controlling cell cycle and regulating apoptosis.
- Myeloprotective actions of inflammatory cytokines TNF-a, TGF-β and Interferons are regulated by activation of p38 MAPK.
- p38 MAPK small molecule inhibitors (SB203580 and SB202190) reverses inhibitory cytokine effects on hematopoietic cells.

Results

- ARRYY-614- Specific dual inhibitor of p38 MAPK and Tie-2 inhibits cytokine-mediated myelosuppressive effects in hematopoietic cell and inhibits hematopoietic cell proliferation. Leukemic cells were treated with SP600125 (5 μM) and SB203580 (10 μM) and assessed for cytokine-mediated effects, cytokine-induced cell proliferation, and apoptosis.
- p38 MAPK small molecule inhibitors (SB203580 and SB202190) reverses inhibitory cytokine effects on hematopoietic cells.